



Središnja medicinska knjižnica

Šagud M., Nikolac Perković M., Vuksan-Ćusa B., Maravić A., Švob Štrac D., Mihaljević Peleš D., Živković M., Kušević Z., Pivac N. (2016) *A prospective, longitudinal study of platelet serotonin and plasma brain-derived neurotrophic factor concentrations in major depression: effects of vortioxetine treatment. Psychopharmacology, 233 (17). pp. 3259-67. ISSN 0033-3158*

<http://www.springer.com/journal/213>

<http://link.springer.com/journal/213>

The final publication is available at Springer via
<https://doi.org/10.1007/s00213-016-4364-0>

<http://medlib.mef.hr/2774>

University of Zagreb Medical School Repository

<http://medlib.mef.hr/>

A prospective, longitudinal study of platelet serotonin and plasma brain-derived neurotrophic factor concentrations in major depression: effects of vortioxetine treatment

Marina Sagud ^{1*}, Matea Nikolac Perkovic ^{2*}, Bjanka Vuksan-Cusa ^{1,3}, Anja Maravic ⁴, Dubravka Svob Strac ², Alma Mihaljevic Peles ¹, Maja Zivkovic ⁴, Zorana Kusevic ¹, Nela Pivac ²

¹ School of Medicine, University of Zagreb, Clinical Hospital Centre Zagreb, Department of Psychiatry and, University of Zagreb, Zagreb, Croatia

² Rudjer Boskovic Institute, Division of Molecular Medicine, Zagreb, Croatia

³ Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

⁴ Clinics for Psychiatry Vrapce, Zagreb, Croatia

* Marina Sagud and Matea Nikolac Perkovic equally contributed to this work

Correspondence to:

Prof. Nela Pivac, PhD, Senior Scientist

Division of Molecular Medicine

Rudjer Boskovic Institute

Bijenicka cesta 54, 10 000 Zagreb, Croatia

Phone: + 385 1 457 1207; Fax: + 385 1 456 1010

E-mail: npivac@irb.hr

Acknowledgements

This study was supported by the University of Zagreb, Project Code: BM126, and the Croatian Psychiatric Association. The authors thank Marin Kirigin, MD, for editing the English language.

Conflict of interest

The authors declare no conflicts of interest in relation to the current study, and in general.

Abstract

Background

Various antidepressants occupy brain serotonin transporter (SERT), decrease platelet serotonin (5-HT) concentration, and normalize reduced plasma brain-derived neurotrophic factor (BDNF) concentrations in depressed patients. Vortioxetine is a recently introduced antidepressant with a multimodal mechanism of action. In addition to SERT inhibition, vortioxetine acts via different 5-HT receptors. To further elucidate its mechanism of action, we have investigated the effects of vortioxetine on platelet 5-HT and plasma BDNF concentrations in patients with major depression.

Methods

Platelet 5-HT and plasma BDNF concentrations were determined in 44 healthy subjects at baseline and in 44 depressed patients before and after 4 weeks of treatment with vortioxetine (5-15 mg daily). Platelet 5-HT concentration was determined using the ortho-phthalaldehyde-enhanced fluorometric method, and plasma BDNF concentration using a commercial enzyme-linked immunosorbent assay (Quantikine ELISA, R&D Systems).

Results

At baseline, platelet 5-HT concentrations did not differ between depressed and control subjects, but plasma BDNF values were lower ($p=0.011$; $\omega=0.80$) in depressed patients than in healthy subjects. Vortioxetine treatment significantly ($p<0.0001$; $\omega=0.80$) decreased platelet 5-HT concentration and significantly ($p=0.004$; $\omega=0.80$) increased plasma BDNF concentration in depressed patients compared to their baseline values. Age, gender, and smoking were not significantly associated with platelet 5-HT and plasma BDNF concentrations.

Conclusion

Despite a novel mechanism of action, vortioxetine shares some common effects with other antidepressants. This study is the first to show that, in addition to clinical improvement, 4 weeks of treatment with vortioxetine (5-15 mg daily), decreased platelet 5-HT and increased plasma BDNF concentrations in depressed patients.

Keywords

Antidepressants, Depression, Plasma BDNF, Platelet serotonin, Vortioxetine

Introduction

Major depressive disorder (MDD) is a highly prevalent and disabling condition, and a leading contributor to the global burden of disease (Global burden of disease study 2013 collaborators 2015). Antidepressants, mainly selective serotonin (5-hydroxytryptamine (5-HT)) reuptake inhibitors (SSRIs) and 5-HT and noradrenaline reuptake inhibitors (SNRIs), are first-line treatment for moderate to severe depressive episodes (Malhi et al. 2013). However, because of the limited efficacy, low remission rates (Rush et al. 2006), and treatment resistant depression (TRD) (Thomas et al. 2013), new treatment strategies are needed (Mihaljevic-Peles et al. 2011). Vortioxetine is a recently introduced antidepressant with a novel mechanism of action. While SSRIs and SNRIs are monoamine transporter inhibitors (Meyer et al. 2004), vortioxetine is an antidepressant with a multimodal mechanism of action. In addition to serotonin transporter (SERT or 5-HTT) inhibition, vortioxetine is also a 5-HT_{3A} and 5-HT₇ receptor antagonist, 5-HT_{1B} receptor partial agonist, and 5-HT_{1A} receptor agonist (Bang-Andersen et al. 2011).

Like SSRIs and SNRIs, vortioxetine shows a high *in vitro* affinity for SERT (Bang-Andersen et al. 2011). However, in contrast to antidepressants such as citalopram, fluoxetine, sertraline, paroxetine, or extended-release venlafaxine, which occupy around 80% of striatal SERT (Meyer et al. 2004), the occupancy of SERT by vortioxetine in rat ventral hippocampus is only 41% (Mørk et al. 2012). It is hypothesized that vortioxetine at therapeutic doses of 5 to 20 mg/day in humans corresponds to 50 to 80% SERT occupancy (Sanchez et al. 2015), and only high doses of 20-30 mg daily are predicted to obtain SERT occupancy comparable to that reached after effective dose of SSRIs. (Stenkrona et al. 2013). Despite lower SERT occupancy, at least in lower and average daily doses, vortioxetine is an effective antidepressant both in short- and long-term studies (Sanchez et al. 2015), exerting its effects through a combination of lower SERT occupancy and complex activities on various 5-HT receptors (Sanchez et al. 2015). Platelets are an attractive target in neurobiological research due to their accessibility and similarities with neurons. Components of the serotonergic system are also located in platelets. SERT facilitates 5-HT transport into platelets, is structurally identical to neuronal SERT, and is encoded by the same gene as neuronal SERT. Antidepressants, such as paroxetine (Muck-Seler et al. 2002), citalopram (Li et al. 2015), sertraline (Pivac et al. 2003), and fluoxetine (Blardi et al. 2002) that highly occupy brain SERT also markedly decrease platelet 5-HT concentrations.

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor involved in neurogenesis and neural plasticity of the brain, influencing the stability of the synapses, as well as the release of neurotransmitters and neuropeptides (Martinowich and Lu 2008). In addition to brain tissue, BDNF is also present in different peripheral tissues, including plasma, blood cells, and mostly platelets. BDNF promotes the growth, survival, differentiation, and function of serotonergic neurons (Martinowich and Lu 2008), while the regulation of BDNF is mediated by alterations in the serotonergic system (Vaidya et al. 1999). There is evidence that both serum and plasma BDNF concentrations are decreased in patients with MDD (Piccinni et al. 2008) and antidepressants are reported to normalize these

abnormalities (Aydemir et al. 2006; Piccinni et al. 2008; Sen et al. 2008). Some research supports ((Sen et al. 2008) the use of increased peripheral levels of BDNF as a screening tool for novel antidepressant agents, while other research opposes ((Brunoni et al. 2014; Buttenschön et al. 2015; Deuschle et al. 2013; Knorr et al. 2015) this proposition. Antidepressants influence both peripheral BDNF and platelet 5-HT concentrations. While decreases in platelet 5-HT concentrations occur exclusively during treatment with SSRIs, increases in peripheral BDNF concentration have been reported after treatment with antidepressants with different mechanisms of action (Sen et al. 2008), such as paroxetine (Yoshimura et al. 2007), sertraline, fluvoxamine (Yoshimura et al. 2014), escitalopram (Aydemir et al. 2006), citalopram (Li et al. 2015), but also mirtazapine (Deuschle et al. 2013), and milnacipran (Yoshimura et al. 2007). It is assumed that antidepressant-induced inhibition of SERT and increased serotonergic transmission via 5-HT₄, 5-HT₆, 5-HT₇ receptor subtypes influences BDNF expression by increasing the BDNF mRNA levels and subsequent BDNF signaling through tropomyosin receptor kinase B (Galter and Unsicker et al. 2000; Martinowich and Lu 2008). Hence, the therapeutic actions of antidepressants may involve adaptive structural and functional changes in synaptic plasticity that may be underlined by changes in the expression, secretion, or downstream functioning of BDNF (Castrén 2004).

To the best of our knowledge, there are no data reporting whether vortioxetine affects platelet 5-HT concentrations or plasma BDNF concentrations in depressed patients. We hypothesized that treatment with vortioxetine for 4 weeks is associated with clinical improvement, increases in plasma BDNF concentrations, and decreases in platelet 5-HT concentrations in patients with depression, and that these effects will be similar to the effects observed after treatment with other SSRIs.

Methods

Subjects

The study included both inpatient and outpatient drug-naïve Caucasian Croatians aged 18 years or older with a diagnosis of first episode or recurrent episode MDD (referred to as depressed patients). Diagnoses were established according to DSM-IV TR criteria, with a minimum score of 18 on the Hamilton Depression Rating Scale (HDRS)-17 items. Out of 51 included patients, 44 depressed patients completed the study. Drop outs were due to the worsening of symptoms ($N=3$), drug discontinuation ($N=2$), and refusal to come to the scheduled visits ($N=2$). Clinical response was defined as a $\geq 50\%$ reduction of baseline HDRS scores. Patients were recruited in the University Hospital Centre Zagreb, and Clinics for Psychiatry Vrapce, Zagreb, Croatia. After an initial interview and baseline assessment, patients received 4 weeks of treatment with vortioxetine. At week 4, the majority of patients were taking 10 mg vortioxetine daily, while 4 patients were taking 5mg, and 3 patients were taking 15 mg. There were no serious adverse events during the study: 3 patients reported nausea, 1 patient reported abdominal pain, and 1 patient reported headache.

The control group comprised 44 healthy drug-naïve Caucasian Croatians, aged 18 years or older, unrelated to the study group. The control group donated blood samples during regular check-ups at the same time as depressed patients.

Before sampling, all subjects filled out a detailed questionnaire about their medical history and smoking habits. All subjects were matched for gender. Exclusion criteria for all subjects were: acute respiratory tract infections; treatment with tryptophan, St John's Wort, antidepressants, mood stabilizers, antipsychotics, estrogen replacement therapy, or opioid analgesics at least one month prior to inclusion (with the exception of fluoxetine, which had to be withdrawn at least 6 weeks prior to study entry); the presence of psychotic symptoms; treatment resistance to the current episode of depression; and diagnosis of a comorbid psychotic disorder, bipolar disorder, severe alcohol or other substance abuse and dependence, obsessive-compulsive disorder, eating disorder, dementia, and severe somatic disorders including poorly controlled arterial hypertension, diabetes and thyroid disease.

Study procedures were approved by the local Ethics Committees and are presented in Table 1. Informed consent was obtained from all participants. All procedures were in accordance with the ethical standards established by the Helsinki Declaration of 1975 (as revised in 1983).

Blood sample collection

Whole blood samples were collected in 8.5 ml yellow-top Vacutainer tubes with 1.5 ml of acid citrate dextrose anticoagulant. Sampling was performed at 8 a.m., following an overnight fast, and was a part of the routine laboratory visits. Platelets were obtained by series of centrifugation of whole blood and later from platelet-rich-plasma. Aliquots of plasma were separated for BDNF analysis and stored at -20 °C. To decrease variability, samples were processed within 1 h of being collected.

Determination of platelet 5-HT concentration

Platelets were obtained from platelet-rich-plasma and then disrupted by sonication. Platelet 5-HT concentrations were determined using ortho-phthalaldehyde (OPT)-enhanced fluorometry. Briefly, standards (5-HT) and platelet sonicates (both in duplicates) were incubated with 10 % ZnSO₄ and 1N NaOH at room temperature for 5 minutes to precipitate proteins. Supernatant was transferred into a new glass tube and, after adding 1% L-cysteine and 0.01% OPT, boiled for 10 min. The reaction was stopped with 1 N NaOH. The fluorescence of samples was measured on a Varian Spectrophotofluorimeter Cary Eclipse, with an exciting wavelength of 345 nm and emitted wavelength of 485 nm. Platelet protein levels were measured by the method of Lowry et al. (1951).

Measurement of plasma BDNF concentration

BDNF concentration in plasma was determined in duplicate using a commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine ELISA,

R&D Systems, Minneapolis, USA). Plasma samples were diluted 1:2 according to the manufacturer's recommendation. Standards and plasma samples were pipetted into 96-well plates, pre-coated with the monoclonal antibody specific for human BDNF. The plate was sealed and incubated for 2 hours at room temperature. A monoclonal antibody, specific for human BDNF, conjugated to horseradish-peroxidase was added to each well. After a 1-hour incubation period at room temperature, the plate was washed 3 times with washing buffer to remove any unbound antibody-enzyme reagent. A substrate solution was added to the wells and incubated for 30 min at room temperature, protected from light. The reaction was terminated by adding 2 N sulfuric acid and the intensity of the color was measured using a microplate reader set to 450 nm with wavelength correction set to 570 nm. The intra- and inter-assay coefficients of variations were less than 10%. The concentrations of samples in each plate were calculated based on a standard curve.

Statistical evaluation

The results were expressed as means \pm standard deviation, and evaluated with Sigma Stat 3.5 (Jandel Scientific Corp., San Jose, California, USA). Normality of data distribution was confirmed with the Kolmogorov-Smirnov test. Student t-test was used to compare data between healthy subjects and depressed patients. Paired t-test was used to compare data within depressed patients subdivided into those before and after treatment. A multiple linear regression analysis was used to check for the influence of age, gender, and smoking on plasma BDNF and platelet 5-HT concentration, both at baseline and after treatment. All tests were two-tailed and corrected α was set at 0.025. G*Power 3 Software was used to determine a priori sample size and actual power. For the t-test (with $\alpha=0.025$; power $(1 - \beta)=0.800$; a large effect size ($\omega=0.80$)), total desired sample size was 52, and the actual sample size was 84. For the paired t-test (with $\alpha=0.025$; power $(1 - \beta)=0.800$; a medium effect size ($\omega=0.30$)), total desired sample size was 34, and the actual sample size was 44. For multiple regression analysis (with $\alpha=0.05$; power $(1 - \beta)=0.800$; a medium effect size ($\omega=0.15$); number of predictors=3), total desired sample size was 43, and the actual sample size was 44. Therefore, we had the appropriate sample size and statistical power to detect significant differences in the studied groups.

Results

Sample characteristics

Demographic and clinical features of patients are presented in Table 2. Healthy control subjects were 49.8 ± 8.3 years old and did not differ in age ($t=1.27$; $df=86$; $p=0.208$; Student's t-test) from depressed patients (Table 2). Regarding smoking, there were no significant differences ($\chi^2=0.056$, $df=1$; $p=0.813$) in the frequency of smokers and nonsmokers between depressed patients and healthy control subjects. As expected, the total HDRS scores of patients significantly ($p=0.0001$) decreased after 4 weeks of vortioxetine treatment compared to baseline scores (Table 2).

Platelet 5-HT and plasma BDNF concentration

Multiple linear regression analyses evaluated the possible influence of age, gender, and smoking status on platelet 5-HT, both at baseline and after treatment. Using platelet 5-HT concentrations as the dependent variable, and age, gender, and smoking as independent variables, the model was not significant before ($F(3, 40)=0.233$; $p=0.873$; $R_{adj}^2=-0.061$) or after ($F(3, 40)=1.867$; $p=0.152$; $R_{adj}^2=0.061$) treatment with vortioxetine. Age ($p=0.960$), gender ($p=0.957$), and smoking ($p=0.414$) did not significantly affect platelet 5-HT concentrations before treatment. After vortioxetine treatment, gender ($p=0.573$) and smoking ($p=0.245$) were not significantly associated with platelet 5-HT concentrations. Since age was marginally associated with platelet 5-HT concentrations ($p=0.039$), after treatment, Spearman's coefficient of rank correlation was used to assess this relationship. It revealed no significant correlation between platelet 5-HT concentrations and age in patients before ($r=0.159$; $p=0.304$) or after ($r=-0.065$; $p=0.673$) treatment.

Multiple linear regression analysis used plasma BDNF concentration as the dependent variable, and age, gender, and smoking as independent variables. It revealed that the model was not significant either at baseline ($F(3, 40)=1.490$; $p=0.233$; $R_{adj}^2=0.035$), or after treatment ($F(3, 40)=2.231$; $p=0.101$; $R_{adj}^2=0.084$). This lack of significant association was due to a lack of significant effect of age ($p=0.723$), gender ($p=0.136$), and smoking ($p=0.088$) on plasma BDNF concentration before treatment, or a lack of significant effect of age ($p=0.213$) and gender ($p=0.259$) on plasma BDNF concentration after vortioxetine treatment. Although the model was not significant, as the significant effect of smoking ($p=0.023$) was detected after treatment, to further evaluate the possible impact of gender and smoking on plasma BDNF concentration all subjects were subdivided according to gender or smoking (Table 3).

Platelet 5-HT and plasma BDNF concentrations did not differ significantly among healthy subjects or among depressed patients before or after treatment, all of whom were subdivided according to smoking status and gender (Table 3). Consequently, in the further analyses subjects were merged according to the gender and according to the smoking status.

Individual data for platelet 5-HT and plasma BDNF concentrations are presented in Figs. 1 and 2. Relative changes in platelet 5-HT and BDNF concentrations after vortioxetine treatment are shown in Table 4.

Platelet 5-HT concentrations were similar ($t=0.308$; $df=86$; $p=0.759$; Student's t-test) between control subjects and depressed patients before treatment (Fig. 1). BDNF concentrations differed significantly ($t=2.594$; $df=86$; $p=0.011$; Student's t-test) between control subjects and depressed patients before treatment, as control subjects had significantly higher plasma BDNF concentrations than depressed patients at baseline (Fig. 2).

Vortioxetine treatment for 4 weeks significantly affected platelet 5-HT concentrations in depressed patients. Platelet 5-HT concentrations were significantly decreased ($t=7.068$; $df=43$; $p<0.0001$, Paired

samples t-test) after 4 weeks of vortioxetine treatment compared to baseline values (Fig. 1). In addition, depressed patients had significantly ($t=6.920$; $df=86$; $p<0.0001$, Student t-test) lower platelet 5-HT concentrations than control subjects after vortioxetine treatment. (Fig. 1).

In depressed patients, plasma BDNF concentration was significantly increased ($t=2.997$; $df=43$; $p=0.0045$, Paired samples t-test) after vortioxetine treatment compared to their baseline plasma BDNF concentration (Fig. 2). This treatment “normalized” plasma BDNF concentration in depressed patients since BDNF concentrations did not differ significantly ($t=0.570$; $df=86$; $p=0.570$) from the BDNF values in control subjects (Fig. 2).

To evaluate (Paired samples t-test) the possible relationship between platelet 5-HT and plasma BDNF concentrations and the first or the recurrent episode of depression, patients were subdivided into first episode and recurrent episode depression. Platelet 5-HT concentrations were similar in patients both with first episode or recurrent episode depression, before ($t=0.516$; $df=42$; $p=0.609$) and after ($t=1.324$; $df=42$; $p=0.193$) vortioxetine treatment. Similarly, plasma BDNF concentration did not differ significantly ($t=1.464$; $df=42$; $p=0.151$) between patients in the first or the recurrent episode of depression, before ($t=-1.464$; $df=42$; $p=0.151$) or after ($t=-0.287$; $df=42$; $p=0.775$) vortioxetine treatment.

To assess the possible correlation between changes in the HDRS scores and plasma BDNF or platelet 5-HT concentrations, and between plasma BDNF and platelet 5-HT, Pearson’s coefficient of rank correlation was used. It revealed no significant correlation between changes in the HDRS scores and plasma BDNF ($r=0.063$; $p=0.698$) or platelet 5-HT ($r=0.231$; $p=0.151$) respectively, or between changes in plasma BDNF and platelet 5-HT concentrations ($r=0.198$; $p=0.204$).

Discussion

To the best of our knowledge, this is the first study that compared platelet 5-HT and plasma BDNF concentrations in depressed patients before and after 4 weeks of treatment with the novel antidepressant vortioxetine (5-15 mg daily). The main findings from the present study are: 1) vortioxetine treatment significantly decreased platelet 5-HT concentration and HDRS scores when compared to values before treatment; 2) this treatment significantly increased plasma BDNF concentration.

According to our results, vortioxetine (5-15 mg/day) decreased platelet 5-HT concentration, similar to the effect of other SSRIs (Blardi et al. 2002; Li et al. 2015; Muck-Seler et al. 2002; Pivac et al. 2003). This finding is in line with vortioxetine’s high SERT affinity *in vitro* (Bang-Andersen et al. 2011), but in slight discordance with only partial SERT occupancy detected in rats (Bang-Andersen et al. 2011) and humans (Areberg et al. 2012). The majority of our patients received 10 mg of vortioxetine daily, and this dose is reported to correspond to 63% of SERT occupancy (Areberg et al. 2012), which was below the SSRI/SNRI efficacy threshold of 80%. Our study is the first to show *in vivo* effects of vortioxetine on platelet 5-HT concentrations in depressed patients, as other studies

have only been conducted on healthy and younger subjects (Areberg et al. 2012; Stenkrona et al. 2013). A meta-analysis of molecular imaging data revealed reductions of around 10% availability in 5-HT reuptake sites in different brain regions in drug-naïve depressed patients compared to healthy subjects (Uebelhack et al. 2006), which were further reduced with increased age (Gryglewski et al. 2014; Rominger et al. 2015). SSRIs have been found to modulate 5-HT concentration in a similar manner in platelets and neurons (Yubero-Lahoz et al. 2013), and theoretically, the higher the central SERT occupancy, the lower the platelet 5-HT concentration. We speculate that in our patients, vortioxetine has sufficiently inhibited SERT in platelets to cause marked decreases in platelet 5-HT concentrations. In line with previous reports (Muck-Seler et al. 2002; Uebelhack et al. 2006), no differences in platelet 5-HT concentrations between depressed patients and healthy controls were found. In our study, and in agreement with previous data (Nenadic Sviglin et al. 2011), platelet 5-HT concentrations were not affected by age and gender, both in healthy controls and depressed patients. This result corresponds with the absence of an association between smoking and platelet 5-HT concentrations in healthy subjects (Klein et al. 2007; Launay et al. 2009) or psychiatric patients (Kovacic et al. 2008).

The present study also detected increased plasma BDNF concentration in depressed patients after 4 weeks of treatment with vortioxetine. Our results are in agreement with reports showing increased plasma BDNF in depressed patients treated with SSRIs such as sertraline (Brunoni et al. 2014), citalopram (Haghighi et al. 2013), fluoxetine or desvenlafaxine (Ghosh et al. 2015), and paroxetine (Yasui-Furukori et al. 2011), and in responders to paroxetine, citalopram and venlafaxine (Lee and Kim 2008). In our study depressed patients had decreased plasma BDNF concentrations compared to healthy subjects, consistent with findings across studies (Fornaro et al. 2015; Lee and Kim 2008; Piccinni et al. 2008; Yoshimura et al. 2007), although these remain limited and insufficient for meta-analysis (Polyakova et al. 2015).

While we investigated the influence of vortioxetine on BDNF concentration in plasma, many studies have determined the effect of antidepressants on serum BDNF concentration. The majority of serum BDNF originates from platelets and therefore, plasma reflects short-term, while serum reflects long-term BDNF content (Polyakova et al. 2015). However, it is still unclear whether plasma and serum BDNF concentrations are interdependent (Polyakova et al. 2015). It has been reported that both plasma and serum BDNF concentrations are decreased in depressed patients, and that plasma BDNF concentration increases, while serum BDNF levels remains low at the 1st, 3rd, 6th and 12th month of antidepressant treatment (Piccinni et al. 2008). Though it is assumed that serum BDNF is stored in human platelets (Fujimura et al. 2002), the source of plasma BDNF is still not fully explained. Plasma BDNF could originate from vascular endothelial and smooth muscle cells (Nakahashi et al. 2000), or it may be released by macrophages and lymphocytes after their activation (Kerschensteiner et al. 1999). There are also indications that BDNF can cross the blood-brain barrier, which is why neurons and glia could also be the source of circulating BDNF (Karege et al. 2002; Pan et al. 1998).

Several limitations in our study should be addressed. Exercise levels that may increase BDNF concentration (Rasmussen et al. 2009) were not determined. Female subjects were not assessed for menopausal status or phase of menstrual cycle. However, no associations were found between plasma BDNF levels and luteinizing hormone, follicle-stimulating hormone, estrogen and progesterone levels (Baek et al. 2014). Furthermore, in our study age and gender were not significantly associated with plasma BDNF. The main advantage of our study is that it is the first in which platelet 5-HT and plasma BDNF concentration were determined simultaneously in depressed patients before and after treatment with a novel antidepressant vortioxetine. Also, our study included only drug-naïve Croatian Caucasians, and the findings revealed significant effects of vortioxetine treatment on platelet 5-HT and plasma BDNF concentrations. The study had an appropriate sample size and the statistical power to detect significant differences between groups.

In conclusion, 4 weeks of treatment with vortioxetine decreased platelet 5-HT and increased plasma BDNF concentrations in patients with MDD. These results suggest that in spite of a novel and distinct mechanism of action, vortioxetine shares some common effects with other antidepressants.

Compliance with ethical standards

Study procedures were approved by the local Ethics Committees. Informed consent was obtained from all participants. All procedures were in accordance with the ethical standards laid out in Helsinki Declaration of 1975 (as revised in 1983). The authors declare that they have no conflicts of interest in relation to the current study and in general.

References

- Areberg J, Luntang-Jensen M, Søgaaard B, Nilausen DØ (2012) Occupancy of the serotonin transporter after administration of Lu AA21004 and its relation to plasma concentration in healthy subjects. *Basic Clin Pharmacol Toxicol* 110:401-404
- Aydemir C, Yalcin ES, Aksaray S, Kisa C, Yildirim SG, Uzbay T, Goka E (2006) Brain-derived neurotrophic factor (BDNF) changes in the serum of depressed women. *Progr Neuropsychopharmacol Biol Psychiatry* 30:1256-1260
- Baek JH, Kang ES, Fava M, Mischoulon D, Nierenberg AA, Lee D, Heo JY, Jeon HJ (2014) Thyroid stimulating hormone and serum, plasma, and platelet brain-derived neurotrophic factor during a 3-month follow-up in patients with major depressive disorder. *J Affect Disord* 169:112-117
- Bang-Andersen B, Ruhland T, Jørgensen M, Smith G, Frederiksen K, Jensen KG, Zhong H, Nielsen SM, Hogg S, Mørk A, Stensbøl TB (2011) Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressed disorder. *J Med Chem* 54:3206-3221
- Blardi P, De Lalla A, Leo A, Auteri A, Iapichino S, Di Muro A, Dell'Erba A, Castrogiovanni P (2002) Serotonin and fluoxetine levels in plasma and platelets after fluoxetine treatment in depressed patients. *J Clin Psychopharmacol* 22:131-136
- Brunoni AR, Machado-Vieira R, Zarate CA Jr, Vieira EL, Vanderhasselt MA, Nitsche MA, Valiengo L, Benseñor IM, Lotufo PA, Gattaz WF, Teixeira AL (2014) BDNF plasma levels after antidepressant treatment with sertraline and transcranial direct current stimulation: results from a factorial, randomized, sham-controlled trial. *Eur Neuropsychopharmacol* 24:1144-1151
- Buttenschön HN, Foldager L, Elfving B, Poulsen PHP, Uher R, Mors O (2015) Neurotrophic factors in depression in response to treatment. *J Affect Disord* 183:287-294
- Castrén E (2004) Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol* 4:58-64
- Deuschle M, Gilles M, Scharnholtz B, Lederbogen F, Lang UE, Hellweg R (2013) Changes of serum concentrations of brain-derived neurotrophic factor (BDNF) during treatment with venlafaxine and mirtazapine: role of medication and response to treatment. *Pharmacopsychiatry* 46:54-58
- Fornaro M, Escelsior A, Rocchi G, Conio B, Magioncalda P, Marozzi V, Presta A, Sterlini B, Contini P, Amore M, Fornaro P, Martino M (2015) BDNF plasma levels variations in major depressed patients receiving duloxetine. *Neurol Sci* 36:729-734
- Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, Sun B, Tandon NN (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost* 87:728-734
- Galter D, Unsicker K (2000) Brain-derived neurotrophic factor and trkB are essential for cAMP-mediated induction of the serotonergic neuronal phenotype. *J Neurosci Res* 61:295-301

- Ghosh R, Gupta R, Bhatia MS, Tripathi AK, Gupta LK (2015) Comparison of efficacy, safety and brain derived neurotrophic factor (BDNF) levels in patients of major depressed disorder, treated with fluoxetine and desvenlafaxine. *Asian J Psychiatr* 18:37-41
- Global burden of disease study 2013 collaborators (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386:743-800
- Gryglewski G, Lanzenberger R, Kranz GS, Cumming P (2014) Meta-analysis of molecular imaging of serotonin transporters in major depression. *J Cereb Blood Flow Metab* 34:1096-1103
- Haghighi M, Salehi I, Erfani P, Jahangard L, Bajoghli H, Holsboer-Trachsler E, Brand S (2013) Additional ECT increases BDNF-levels in patients suffering from major depressed disorders compared to patients treated with citalopram only. *J Psychiatr Res* 47:908-915
- Karege F, Schwald M, Cisse M (2002) Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* 328:261–264
- Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H, Hohlfeld R (1999) Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med* 189:865–870
- Klein N, Sacher J, Geiss-Granadia T, Mossaheb N, Attarbaschi T, Lanzenberger R, Spindelegger C, Holik A, Asenbaum S, Dudczak R, Tauscher J, Kasper S (2007) Higher serotonin transporter occupancy after multiple dose administration of escitalopram compared to citalopram: an [¹²³I]ADAM SPECT study. *Psychopharmacology (Berl)* 191:333-339
- Knorr U, Koefoed P, Soendergaard MH, Vinberg M, Gether U, Gluud C, Wetterslev J, Winkel P, Kessing LV (2015) No effect of escitalopram versus placebo on brain-derived neurotrophic factor in healthy individuals: a randomised trial. *Acta Neuropsychiatr* 28:101-109
- Kovacic Z, Henigsberg N, Pivac N, Nedic G, Borovecki A (2008) Platelet serotonin concentration and suicidal behavior in combat related posttraumatic stress disorder. *Progr Neuropsychopharmacol Biol Psychiatry* 32:544-551
- Launay JM, Del Pino M, Chironi G, Callebert J, Peoc'h K, Mégnien JL, Mallet J, Simon A, Rendu F (2009) Smoking Induces Long-Lasting Effects through a Monoamine-Oxidase Epigenetic Regulation. *PLoS ONE* 4:e7959
- Lee HY, Kim YK (2008) Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants. *Neuropsychobiology* 57:194-199
- Li X, Fan Y, Xiao S, Peng S, Dong X, Zheng X (2015) Decreased platelet 5-hydroxytryptamin (5-HT) levels: a response to antidepressants. *J Affect Disord* 187:84-90

- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-275
- Malhi GS, Hitching R, Berk M, Boyce P, Porter R, Fritz K (2013) Pharmacological management of unipolar depression. *Acta Psychiatr Scand Suppl* 443:6-23
- Martinowich K, Lu B (2008) Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* 33: 73-83
- Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ, Ginovart N, Spencer EP, Cheek A, Houle S (2004) Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: An [¹¹C]DASB positron emission tomography study. *Am J Psychiatry* 161:826–835
- Mihaljevic-Peles A, Sagud M, Janovic MB, Mikulic SK, Jevtovic S (2011) Do we need new therapeutic strategies for depression? *Psychiatr Danub* 23:300-301
- Mørk A, Pehrson A, Brennum LT, Nielsen SM, Zhong H, Lassen AB, Miller S, Westrich L, Boyle NJ, Sánchez C, Fischer CW, Liebenberg N, Wegener G, Bundgaard C, Hogg S, Bang-Andersen B, Stensbøl TB (2012) Pharmacological effects of Lu AA21004: a novel multimodal compound for the treatment of major depressed disorder. *J Pharmacol Exp Ther* 340:666-675
- Muck-Seler D, Pivac N, Sagud M, Jakovljevic M, Mihaljevic-Peles A (2002) The effects of paroxetine and tianeptine on peripheral biochemical markers in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 26:1235-1243
- Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, Sun B (2000) Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett* 470:113–117
- Neadic Svirgin K, Nedic G, Nikolac M, Mustapic M, Muck-Seler D, Borovecki F, Pivac N (2011) Insomnia, platelet serotonin and platelet monoamine oxidase in chronic alcoholism. *Neurosci Lett* 500:172-176
- Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ (1998) Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 37:1553–1561
- Piccinni A, Marazziti D, Catena M, Domenici L, Del Debbio A, Bianchi C, Mannari C, Martini C, Da Pozzo E, Schiavi E, Mariotti A, Roncaglia I, Palla A, Consoli G, Giovannini L, Massimetti G, Dell'Osso L (2008) Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. *J Affect Disord* 105:279-283.
- Pivac N, Muck-Seler D, Sagud M, Jakovljevic M, Mustapic M, Mihaljevic-Peles A (2003) Long-term sertraline treatment and peripheral biochemical markers in female depressed patients. *Prog Neuropsychopharmacol Biol Psychiatry* 27:759-765
- Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML (2015) BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. *J Affect Disord* 174:432-440

- Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H (2009) Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* 94:1062–1069
- Rominger A, Cumming P, Brendel M, Xiong G, Zach C, Karch S, Tatsch K, Bartensteina P, la Fougèree C, Kocha W, Pogarellc O (2015) Altered serotonin and dopamine transporter availabilities in brain of depressed patients upon treatment with escitalopram: A [123 I] β -CIT SPECT study. *Eur Neuropsychopharmacol* 25:873-881
- Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. *Am J Psychiat* 163:1905–1917
- Sanchez C, Asin KE, Artigas F (2015) Vortioxetine, a novel antidepressant with multimodal activity: review of preclinical and clinical data. *Pharmacol Ther* 145:43-57
- Sen S, Duman R, Sanacora G (2008) Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry* 64:527-532
- Stenkrona P, Halldin C, Lundberg J (2013) 5-HTT and 5-HT(1A) receptor occupancy of the novel substance vortioxetine (Lu AA21004). A PET study in control subjects. *Eur Neuropsychopharmacol* 23:1190-1198
- Thomas L, Kessler D, Campbell J, Morrison J, Peters TJ, Williams C, Lewis G, Wiles N (2013) Prevalence of treatment-resistant depression in primary care: cross-sectional data. *Br J Gen Pract* 63:e852-858
- Uebelhack R, Franke L, Herold N, Plotkin M, Amthauer H, Felix R (2006) Brain and platelet serotonin transporter in humans-correlation between [123 I]-ADAM SPECT and serotonergic measurements in platelets. *Neurosci Lett* 406:153-158
- Vaidya VA, Terwilliger RM, Duman RS (1999) Role of 5-HT_{2A} receptors in the stress-induced down-regulation of brain-derived neurotrophic factor expression in rat hippocampus. *Neurosci Lett* 262:1-4
- Yasui-Furukori N, Tsuchimine S, Nakagami T, Fujii A, Sato Y, Tomita T, Yoshizawa K, Inoue Y, Kaneko S (2011) Association between plasma paroxetine concentration and changes in plasma brain-derived neurotrophic factor levels in patients with major depressed disorder. *Hum Psychopharmacol* 26:194-200
- Yoshimura R, Kishi T, Hori H, Katsuki A, Sugita-Ikenouchi A, Umene-Nakano W, Atake K, Iwata N, Nakamura J (2014) Serum Levels of Brain-Derived Neurotrophic Factor at 4 Weeks and Response to Treatment with SSRIs. *Psychiatry Investig* 11:84-88
- Yoshimura R, Mitoma M, Sugita A, Hori H, Okamoto T, Umene W, Ueda N, Nakamura J (2007) Effects of paroxetine or milnacipran on serum brain-derived neurotrophic factor in depressed patients. *Prog. Neuropsychopharmacol. Biol Psychiatry* 31:1034-1037

Yubero-Lahoz S, Robledo P, Farré M, De Latorre R (2013) Platelet SERT as a peripheral biomarker of serotonergic neurotransmission in the central nervous system. *Curr Med Chem* 20:1382-1396

Table 1 Study procedures for patients with major depressive disorder

Procedure	Baseline	Week 4
Initial interview, Inclusion/exclusion criteria, Signing ICD, Demographic data	x	
HDRS-17	x	x
Platelet serotonin concentration	x	x
Plasma BDNF concentration	x	x

BDNF, brain-derived neurotrophic factor; HDRS, Hamilton Rating Scale for Depression

Table 2 Demographic and clinical data of patients with major depressive disorder

	<i>N</i> =44
Sex (male/female)	14/30
Smoking (yes/no)	12/32
Depressive episode (first or single/recurrent)	22/22
Family history of depression (yes/no)	15/29
Suicide attempt (yes/no)	3/41
Age	52.9 (13.7)
HDRS score:	
at baseline	30.3 (5.6)
after treatment	11.9 (7.1) ^a

Values for age and HDRS score are presented as mean (standard deviation)

^a vs HDRS baseline score ($t=-21.33$; $df=40$; $p<0.0001$)

BDNF, brain-derived neurotrophic factor; HDRS, Hamilton Rating Scale for Depression

Table 3 Platelet serotonin concentrations and concentration of plasma brain-derived neurotrophic factor (BDNF) in healthy subjects and patients with major depressive disorder (evaluated before or after vortioxetine treatment) subdivided according to smoking status and gender

	Platelet serotonin			Plasma BDNF		
	Healthy subjects	Depressed subjects		Healthy subjects	Depressed subjects	
		At baseline	After treatment		At baseline	After treatment
Smoking						
Yes	1.59 (0.74)	1.29 (0.48)	0.62 (0.34)	0.56 (0.29)	0.51 (0.43)	0.87 (0.82)
No	1.37 (0.61)	1.47 (0.63)	0.71 (0.33)	0.58 (0.32)	0.36 (0.25)	0.53 (0.32)
Student's t-test (df=42)	$t=-1.02; p=0.316$	$t=0.86; p=0.398$	$t=0.85; p=0.404$	$t=0.20; p=0.842$	$t=-1.42; p=0.163$	$t=-1.94; p=0.059$
Sex						
Male	1.37 (0.60)	1.40 (0.61)	0.71 (0.39)	0.62 (0.32)	0.33 (0.24)	0.55 (0.25)
Female	1.47 (0.68)	1.40 (0.60)	0.67 (0.29)	0.55 (0.31)	0.44 (0.32)	0.66 (0.60)
Student's t-test (df=42)	$t=0.50; p=0.622$	$t=0.01; p=0.993$	$t=-0.34; p=0.737$	$t=-0.71; p=0.483$	$t=1.15; p=0.256$	$t=0.34; p=0.527$

Values are presented as mean (standard deviation)

Table 4 Changes in platelet serotonin and plasma brain-derived neurotrophic factor (BDNF) concentrations in patients with major depressive disorder after vortioxetine treatment, compared to measurements before treatment. Data are shown as the percentage change (%) from baseline.

Sample ID	Δ platelet serotonin	Δ plasma BDNF
1	83.9	499.4
2	7.6	-78.7
3	57.1	1245.8
4	59.6	455.7
5	72.1	1867.3
6	73.3	172.9
7	51.5	729.2
8	42.5	-73,1
9	43.8	20,6
10	54.4	32,8
11	59.2	133.4
12	57.4	30.5
13	52.6	31.8
14	77.5	-5.0
15	23.4	69.6
16	45.2	208.2
17	-46.5	13.0
18	73.8	-37.6
19	29.7	900.2
20	-70.5	135.2
21	38.4	-25.3
22	5.8	192.2
23	48.3	16.3
24	59.2	801.7
25	36.9	54.1
26	71.7	-28.1
27	-49.4	47.1
28	63.4	-76.3
29	9.4	186.1
30	59.3	44,4
31	77.9	-32.6
32	-108.7	-20.8
33	83.1	-47.1
34	65.8	-23.7
35	73.6	194.0
36	42.2	68.4
37	75.1	272.0
38	8.9	11.7
39	47.1	-25.6
40	37.4	190.4
41	12.2	213.8

42	82.6	164.9
43	21.3	26.3
44	72.7	-3.6

Fig. 1 Dot-plot of platelet serotonin (5-HT) concentration in healthy subjects and depressed patients at baseline and after 4 weeks of treatment with vortioxetine. Bold line and whiskers represent mean \pm 1 standard deviation.

* $p < 0.0001$ vs. patients at baseline (Paired samples t-test); # $p < 0.0001$ vs. control subjects (Student t-test)

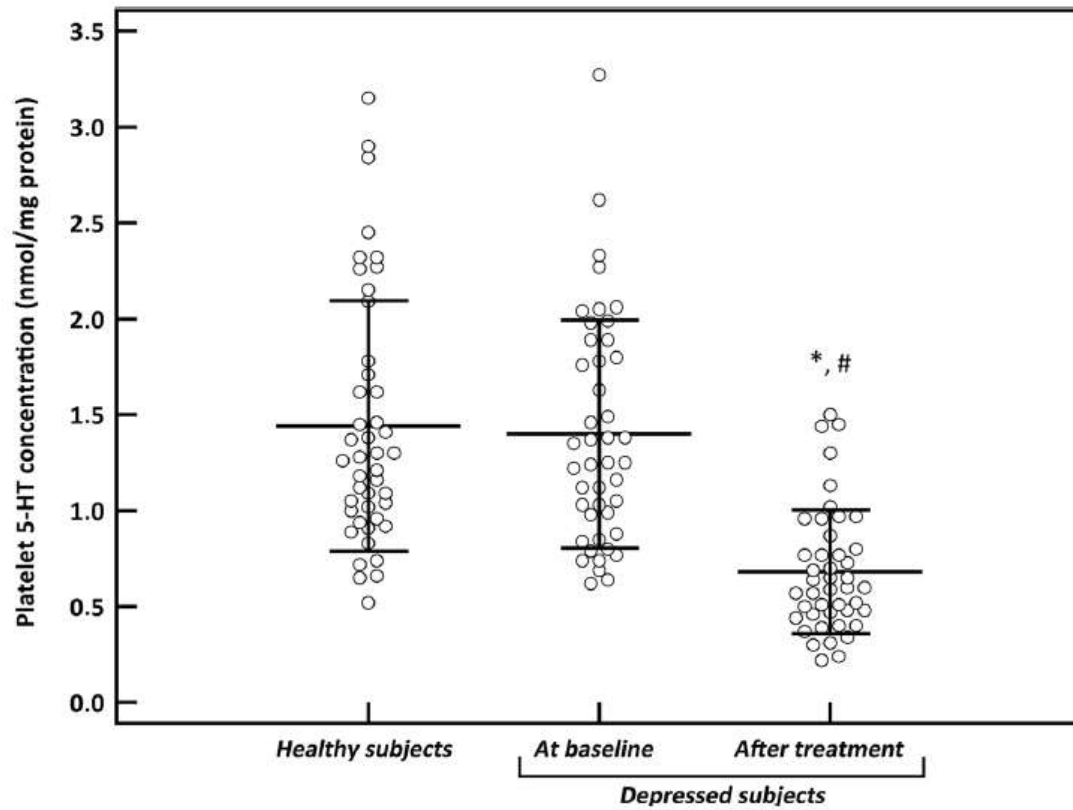


Fig. 2 Dot-plot of plasma brain-derived neurotrophic factor (BDNF) concentration in healthy subjects and depressed patients at baseline and after 4 weeks of treatment with vortioxetine. Bold line and whiskers represent mean \pm 1 standard deviation.

* $p=0.0045$ vs. patients at baseline (Paired samples t-test); # $p<0.0001$ vs. control subjects (Student t-test)

